THE EFFECT OF ADRENIN UPON THE RATE OF LOCOMOTION OF PLANARIA AND OF TOAD LARVÆ.

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In his investigation of the chromaffine system of annelids, Gaskell (2) has pointed out the close morphological and physiological relationship which exists between the chromaffine, the sympathetic and the contractile vascular systems, wherever these are found. He holds that the presence of any systems corresponding physiologically to the first two, necessarily implies the presence of the third on which to act; and in support of this view, he finds in the annelid group, in those forms which possess a contractile vascular system, a cell which he considers the common ancestor of the sympathetic and chromaffine systems. If, therefore, the earliest indication of chromaffine tissue is found among the annelida, it may be safely assumed that in the platyhelminth group in which there is no contractile vascular system, there is nothing comparable to the adrenin-producing cells of higher forms. The effect of adrenal extract is not, however, confined to the regulation of the cardio-vascular apparatus, although this seems to be one of its chief functions. Elliott (1) has definitely shown that it affects other forms of smooth muscle in a manner similar to the sympathetic nervous system, causing either an excitation or an inhibition, according to the nature of the sympathetic fibers supplying the tissue. Its influence is, furthermore, not even limited to smooth muscle for skeletal muscle is affected, also, as various investigators have demonstrated. Is it possible, then, that this secretion of the adrenal medulla exercises an influence upon the contracility of the simple muscles of an organism such as *Planaria*, which does not itself manufacture adrenin?

Bands of circular, longitudinal and diagonal muscles are found in the body wall of planarians; and it is by means of these that locomotion is made possible. It had been generally believed that only the crawling movement is accomplished through muscular contractions, and that gliding results chiefly from the beating of ventral cilia; but Stringer (8) has shown that when muscular activity is entirely checked locomotion does not occur, even though the cilia are beating vigorously; and she concludes that the "locomotion of planarians is essentially a muscular act in which the cilia play no necessary part."

Although the influence of adrenin upon the muscular contractility of *Planaria* cannot be exactly homologized with that upon vertebrate muscle, it is well to bear in mind the results of these investigations. Oliver, and Schäfer (7) report both a greater and a more prolonged contraction of the gastrocnemius in frogs as the result of adrenin injection, and also an increase in muscular activity following administration of the extract by mouth. Takajasu (9), however, working upon isolated muscle, obtained just the opposite effect. He found that the curve of contraction in skeletal muscle, immersed in solutions of adrenin, varying from 1 : 500,000 to 1 : 5,000, becomes successively lower and of shorter duration, although there occurs a preliminary period during which the contractions are slightly prolonged. Gruber (3 and 4) has obtained interesting results with muscle preparations in which fatigue has been induced either electrically or chemically. In these cases, adrenin, instead of causing a still further lowering of the fatigue curve, really produces a rapid recovery of the normal irritability of the muscle. Unlike Oliver and Schäfer, however, he reports no improvement in muscles not fatigued. These investigators, it is evident, have employed various means of introducing the extract, and have worked upon both entire animals and isolated muscle. With planarians, injection is impracticable, and the immersion of isolated muscle altogether impossible; but by placing entire individuals in solutions of adrenin, a combination of the methods of immersion and feeding is obtained.

The experiments here described were carried out upon *Planaria doratocephala*, and *Planaria velata*. The worms were placed in large finger bowls, containing spring water, and kept in the laboratory at as nearly constant temperature as possible. Food consisting of finely minced earthworms of raw meat, was supplied two or three times a week, and at each feeding the water was changed. The Parke, Davis, and Company preparation of adrenalin chloride[1] was at first used; but it was found unsatisfactory, as animals immersed in solutions varying from 1 : 1,000 to 1 : 20,000 soon became inactive, and died within less than an hour. Solutions of the same strength, however, prepared from adrenin in tablet or in crystal form did not produce the same effect; and it seemed not improbable that it was the chloretone in the first preparation which caused the rapid loss of activity. To test this hypothesis, a solution of chloretone was prepared, equal in strength to that contained in the adrenalin chloride preparation, and a comparison was made of the effects upon *Planaria* of similar dilutions of these two. In both cases complete loss of locomotor power quickly resulted. The chloretone alone (1 : 2,000) was sufficient to cause within an hour, a noticeable elongation and lack of response to mechanical stimuli, an effect which is not surprising, since chloretone of 1 : 1,000 strength is recommended as a ready anæsthetic for planarians (10).

In the following experiments, therefore, adrenin in tablet and in crystal form was used. In order to determine whether or not this extract exercises any influence whatever upon *Planaria*, active individuals were put into small bottles containing solutions of adrenin of different strengths, and subjected to the influence of the extract for varying periods of time. It was necessary to keep the bottles corked to prevent the rapid oxidation of the adrenin, but there was enough oxygen present to sustain the worms; and the fact that planarians in corked bottles containing water only were not affected seems proof that the results noted cannot be attributed to lack of oxygen. In the preliminary experiments it was found that animals in solutions of adrenin, varying from 1 : 1,000 to 1 : 15,000 died in less than three days, but in more dilute solutions they remained alive and active even after a week. Most of the later experiments were, therefore, made with a 1 : 15,000 solution. for one day, or with stronger solutions for a shorter period.

[1] Each fluid ounce of this preparation contains $\frac{2}{5}$ grain of adrenalin chloride and $2\frac{1}{4}$ grains of chloretone.

It was difficult to find a means of measuring the influence of the extract upon the musculature. After unsuccessful attempts to record the effect through electrical stimulation, a method of testing muscular activity by means of the rate of locomotion was adopted. A narrow trough, 6.5 cm. long by .3 cm. in width, was made by cementing two ordinary glass slides, side by side, upon a glass plate. Planarians, put into the trough with a little water, readily progressed from one end to the other, usually in the angle formed by the bottom plate and the edge of one slide. A starting place was then marked off; and with a stop-watch the rate of travel was measured. Only continuous non-stop journeys, from the time the head passed the starting point until it reached the end of the trough, were recorded. A large variation was found both in successive rates of the same individual, and in rates of different normal individuals; but by taking the average of a number of successive rates for each animal, and by using the same ones both before and after treatment with adrenin, it was possible to secure results which seem to be significant.

The animals were handled as far as possible in the same way in each experiment; and to avoid differences due to the influence of light, the trough was placed on the table in such a position that the animals moved away from the window in a direction parallel with the entering rays of light. Parker and Burnett (6) have shown that planarians have a "tendency to turn away from the course when directed toward the source of light," and this was easily verified, as it was found almost impossible to keep a worm traveling toward the window. In eighteen successive trials only once did an individual finish the whole length toward the light without reversing; but in the other direction full trips were readily obtained.

In testing the effect upon locomotion, the normal rates of the planarians in water were first recorded, then the rates immediately after exposure to the influence of adrenin, and finally the rates after recovery in water. In the first series (Table I.) ten individuals were used. Only two rates for each were taken under each of the three conditions; but a comparison of the averages of the twenty rates thus obtained indicates an effective influence of adrenin upon the locomotor activity. The average normal rate was 53.4 seconds; but after one day in adrenin solution (1 : 15,000) the average time needed to travel the same distance was increased to 69.5 seconds. Furthermore, as an indication that the change was in response to a definite stimulation, and not merely the expression of a generally weakened physiological condition, such as does occur after a time when the animals are kept in the laboratory, there was a decided improvement after the animals had been placed in water again for two days. The rate this time was even better than the original, 51 seconds.

This same retarding influence of adrenin, followed by recovery in water was found in the next five planarians tested. But the third Series, as is shown in Table I., gave apparently very disappointing results. It may be interesting, however, to note that the animals of Groups B and C which gave good initial rates had been tested before with adrenin. The very poor rates, therefore, of the planarians of Groups A and D, which had been kept for some time in the laboratory, and had not been previously used, indicate that these animals were no longer in the same condition as those of the first experiments and might well be affected differently by the same extract.

Fresh planarians were then obtained and the same tests repeated. The results of these, also recorded in Table I., seem to confirm the conclusion drawn from the first experiments, that adrenin produces a decrease in the normal rate of locomotion of *Planaria*, which is overcome when the animals are again placed in water.

The nature of the effect of adrenin upon the muscular contraction of *Planaria* is not, however, as simple as the decrease in the rate of locomotion would indicate. Observation of the animals reveals clearly that the effect is not merely one of weakened muscular response to mechanical and contact stimuli. There seems to be no delay in the time of reaction to stimulation, and no constant decrease in the strength of the contraction. It is moreover, apparently not the same effect as that which Moore (5) found produced by strychnine, as no reversal of the normal response to stimulation was observed at any time after treatment with adrenin. Experimental animals, touched with a camel's hair brush, reacted in the usual manner by contracting the longitudinal and relaxing the circular and transverse muscles. The effect was rather one of excitement, confined chiefly to the anterior end of the body, which seemed to make coördinated

TABLE I.

Series.	Strength of Sol.	Number of Animals.	Time Exposed to Sol.	No. of Rates Taken.	Average Initial Rate in Sec.	Average Rate in Adrenin.	Average Rate After Return to Water, 1–3 Days.
1.	1 : 15,000	10	24 hrs.	2	53.4	69.5	51
2.	1 : 15,000	5	19 hrs.	2	about 55.	84	59
3. A	1 : 15,000	4	24 hrs.	2	126.5	86.6	66.6
B	1 : 15,000	3	24 "	2	59.2	73.8	64.2
C	1 : 15,000	3	24 "	2	51.5	47.5	60.8
D	1 : 15,000	2	24 "	2	187.	85.5	103.5
E	1 : 15,000	3	22 "	5	69.	67.3	——
4. A	1 : 2,000	1	15 min.	5	91.8	139.8	131
B	1 : 2,000	1	20 "	5	71.6	206	137.5
C	1 : 20,000	3	30 "	5	41.1	48.2	37.2
D	1 : 10,000	4	20 "	5	33.1	52.2	44.3
E	1 : 10,000	4	20 "	5	33.1	51.2	41
F	1 : 10,000	2	20 "	5	34.8	50.9	30.4
G	1 : 10,000	2	20 "	5	30.6	38.7	25.7

locomotion difficult. The animals expended their energy in excited movements of the head, and occasionally were unable even to proceed the whole length of the track. When continuous trips were obtained, a decrease in rate was found, as has been noted; but this cannot be entirely due to a decrease in muscular activity, as there is frequently clear evidence of an increased irritability. At other times, however, the excitatory effect was not noted, and the slower rate was really indicative of a slower rhythmic contraction as the animal progressed along the trough.

This variability of result, expressed as it is in both cases by a decrease in rate, is difficult to explain; and it may be that an explanation is superfluous, and adrenin is not specific in its influence upon planarian muscle. It is possible, however, that we are dealing here with a definite action of the extract; and that a more detailed study of the effect upon the three kinds of muscles, and a more accurate determination of the physiological condition of the individuals at the time they are tested may throw more light on the subject. A possible explanation may be found in the duration of the influence of the extract. The animals which showed to a greater extent the symptoms of excitement were those which had been treated with adrenin for an hour or less. Those which were in the solution for a day, with the exception of some of the groups of Table I., Series 3, showed almost uniformly a real slowing of the rate of muscular contraction. It may be that the initial effect produced by adrenin upon the muscular apparatus of planarians is excitatory in nature, and that the increased irritability, expressed in useless and non-coördinated movements really results in a decrease in the rate of locomotion. As the animals are left in the solution, however, the exciting effect wears off, and there results either an approximate return to normal, as in Series 3, Groups C and E, or a decrease in irritability, following naturally the undue excitement created at first.

This hypothesis of an initial excitatory effect might account also, for some of the other discrepancies of Series 3. The animals of Groups A and D, which showed such a poor initial rate in water may have been in a condition of lowered vitality, perhaps a state of fatigue. Here, as the results show, there followed an increased rate after treatment with adrenin. Might it not be possible that the excitatory effect of the extract was, in these cases, merely sufficient to raise the muscular tone to normal, without producing any symptoms of over-excitation? It is not unlikely that in these animals the increase in the rate of locomotion indicates the real effect of adrenin, which in most of the other experiments is marked by the decrease in rate due to the useless movements of excitement. This possibility may be lessened, perhaps, by the fact that the animals of Group A continued to improve even more after return to water, while those of Group D fell back, indicating that other factors were at work; but this does not exclude the view that adrenin produces a temporary improvement which may or may not become more lasting, dependent upon these other factors.

In order to determine whether or not the same results are produced by repeated doses of adrenin, several further tests were made, the results of which are recorded in Table II. After each treatment with the extract, one to three days in water were allowed for recovery before the next tests were made.

Such variable results were obtained, however, that it is impossible to say whether there is a tendency for *Planaria* to become less sensitive to repeated doses or not. By looking over the percentages of decrease it may be seen that eleven of the twelve animals responded to a lesser degree to the second treatment than to the first; but there is one great exception, and there is, in addition, the result, not recorded in the table, of a third test made with the last series where the decrease was 47 per cent., less than the first but greater than the second. Such variation was to be expected, from the nature of the problem, since the difference in the rate of locomotion does not express the whole of the effect. Although these last experiments add nothing new to our knowledge of planarian reaction to adrenin, they do distinctly confirm the fact that adrenin produces a slowing of the rate of locomotion through an over-excitability which expends itself to no effect.

TABLE II.

Series.	Strength of Sol.	No. Animals Used.	Time Exposed to Sol., Min.	No. Rates Taken.	Ave. Initial Rate in Sec.	First Adrenin, Rate.	Per Cent. Decrease.	Ave. Rate After Return to Water, 1–3 Days.	Second Adrenin, Rate.	Per Cent. Decrease.	Ave. Rate After Return to Water, 1–3 Days.
1	1:10,000	4	20–30	5	40	56.15	40.3	34.8	45.4	30.4	33.2
2	1:10,000	3	25–45	5	29.46	47.35	60.7	33.8	43.8	29.6	38.4
3	1:10,000	1	20	5	45.2	43.7	−03.3	36.2	60	65	53
4	1:10,000	4	20	3	38.4	64.7	68	45.3	57.9	27	41.9

The innervation of the muscles of *Planaria* has not yet been worked out, but it is probable, from the simple character of the nervous system, that the action of adrenin is directly upon the muscle and not through the nerve endings. This point, however, could not be successfully tested. In an attempt to ascertain the rôle of the nervous system it seemed desirable to compare the reactions of the anterior ends of the animals with those of the brainless parts, when exposed to the same solutions of adrenin. A number of individuals were cut in two just posterior to the brain region, and the wounds allowed to close over. It was practically impossible, however, to obtain any satisfactory rates from these pieces even in water, as the small head ends could not be kept in a straight path, and the larger posterior parts moved too slowly in the beginning for any effect of adrenin to be noticeable. It may be noted in this connection that Gruber (3) obtained the same results with denervated vertebrate muscle as with normal nerve-muscle preparations.

Before concluding it may be of interest to mention the results of a few experiments upon toad larvæ. Only fifteen tadpoles were used; and although the results obtained are by no means conclusive they indicate several possibilities which may lead to further investigation. Tadpoles of two ages were used, measuring during the period of experimentation from 8 to 10, and 14 to 16 mm. in length. The rates of locomotion were determined by the time needed to swim around a circular track .7 cm. wide and 25 cm. in circumference. This method was suggested by the habit frequently observed in normal tadpoles of swimming around the sides of the bowls in which they are kept.

Very young tadpoles seemed unaffected by a solution of adrenin crystals 1 : 10,000, although older ones showed some response. Solutions of 1 : 1,000 strength were therefore used, and a slight decrease in rate was obtained. As with *Planaria*, this slowing was again due to a greatly increased excitability which made sustained action impossible. After exposure to the extract the tadpoles reacted spasmodically to mechanical stimulation, swimming about in a highly excited state for a short time; but they soon become exhausted and unable to react until after a period of rest. This state of excitability followed by exhaustion was more pronounced in the larger than in the smaller tadpoles, probably indicating an increased sensitivity.

Adrenalin chloride of the same strength had a very powerful effect, producing absolute paralysis of movement in less than a minute. If the animals were removed at once, however, and allowed to recover in water for an hour, the same excitability and lack of endurance was noted as with the solution of adrenin crystals. This seems to bear out the suggestion made at the beginning that it is the chloretone in the preparation which causes the immediate stupefying effect. The same paralysis, moreover, is produced by a solution of chloretone of strength equal to that contained in the adrenalin chloride solution.

With a less concentrated solution of adrenalin chloride, 1 : 10,000, the same effect was produced. At the end of an hour the animals appeared dead; but after another hour in water, the paralyzing effect wore off and they became very active, but unable to keep up any sustained swimming movements. Kept in water for several days, however, the animals, with one exception, completely recovered, and traveled once more at their normal rate. Adrenin, therefore, seems to have an effect upon larval vertebrate muscle as well as upon the simpler muscle of planarians.

This work was carried out under the direction of Dr. Florence Peebles, to whom I wish now to express my sincere appreciation.

Summary.

1. There is a decrease in the rate of locomotion of planarians after treatment with solutions of adrenin, followed by a return to the normal rate when the animals are again placed in water.

2. In those individuals which have not been subjected to the influence of the extract more than an hour, this decrease in the rate of locomotion seems to be due rather to an increased excitability and lack of coördinated movement than to a slowing of the rate of muscular contraction.

3. Planarians exposed to the action of adrenin for a longer period do not give evidence of extreme excitability, but seem to show a real decline in activity, which may be a secondary effect following the initial excitation.

4. Those planarians whose initial rate indicates a state of depression travel more rapidly after treatment with adrenin. This would seem to confirm the suggestion that the effect of this extract upon planarian muscle is predominantly excitatory.

5. Although no definite statement can be made concerning the effects of adrenal extract upon the muscular contraction of toad larvæ, the indications are that they are similar to the effects produced upon the muscular activity of planarians, and that this effect increases with the age of the individuals.

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